

WHAT IS CLAIMED IS:

1                   1.       An oligonucleotide primer comprising in the following order from 5'  
2 to 3':  
3                   a phage-encoded RNA polymerase recognition sequence,  
4                   a spacer sequence comprising a sequence of from 12 to 20 nucleotides  
5                   that consists of one nucleotide type or two different nucleotide  
6                   types, and  
7                   a target complementary sequence which can bind a segment of a target  
8                   nucleic acid.

1                   2.       The primer of claim 1, wherein said spacer sequence comprises a  
2 nucleotide sequence having the formula  $(XY)_n$ ,  
3 wherein n is from 6 to 10,  
4 wherein X and Y are independently selected from the group consisting of an  
5 adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a  
6 thymidine nucleotide,  
7 wherein X and Y are not the same.

1                   3.       The primer of claim 2, wherein X is an adenine nucleotide and Y is a  
2 guanine nucleotide.

4.       The primer of claim 3, wherein n is 9.

1                   5.       The primer of claim 1, wherein said spacer sequence comprises a  
2 nucleotide sequence having the formula  $(X)_n$ ,  
3 wherein n is from 12 to 20,  
4 wherein X is selected from the group consisting of an adenine nucleotide, a  
5 guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide.

1                   6.       The primer of claim 5, wherein n is 18.

1                   7.       A method of amplifying a target nucleic acid in an aqueous solution  
2 with a first and a second primer, said method comprising:

3                   i.)       transcribing an intermediate duplex with a phage-encoded RNA  
4 polymerase to form a sense transcription product having a 5' end and a 3' end,

wherein said intermediate duplex comprises a double-stranded molecule, wherein said double-stranded DNA molecule comprises a first and a second strand, wherein said first strand comprises in the following order from 5' to 3':  
 a phage-encoded RNA polymerase recognition sequence,  
 a first spacer sequence comprising a sequence of from 12 to 20 nucleotides that consists of one nucleotide type or two different nucleotide types, and  
 a first target complementary sequence which can bind to a segment of said target nucleic acid,  
 wherein said second strand comprises in the following order from 5' to 3':  
 a second target complementary sequence which can bind to a segment of said target nucleic acid,  
 a second spacer sequence comprising a sequence of from 12 to 20 nucleotides that consists of one nucleotide type or two different nucleotide types, and  
 a phage-encoded RNA polymerase recognition sequence,  
 wherein said transcribing takes place in the presence of  $Mn^{++}$ , with all four dNTPs, and with those rNTPs represented in said first spacer sequence;

ii.) hybridizing said second primer to said sense transcription product to form a second primer-sense transcription product complex,  
 wherein said second primer comprises in the following order from 5' to 3':  
 a phage-encoded RNA polymerase recognition sequence,  
 said second spacer sequence, and  
 said second target complementary sequence which can bind to a 3' segment of said target nucleic acid;

iii.) extending said second primer-sense transcription product complex with a Reverse Transcriptase that lacks RNaseH activity to form a first amplification duplex;

iv.) transcribing said first amplification duplex with a phage-encoded RNA polymerase, in the presence of  $Mn^{++}$ , with all four dNTPs, and with those rNTPs represented in said second spacer sequence, to form an antisense transcription product;

- 39 v.) hybridizing said first primer to said antisense transcription product to  
40 form a first primer-antisense transcription product complex,  
41 wherein said first primer comprises in the following order from 5' to  
42 3':  
43 a phage-encoded RNA polymerase recognition sequence,  
44 said first spacer sequence, and  
45 said first target complementary sequence which can bind to a  
46 5' segment of said target nucleic acid;
- 47 vi.) extending said second primer-antisense transcription product complex  
48 with a Reverse Transcriptase that lacks RNaseH activity to form a second amplification  
49 duplex; and
- 50 vii.) transcribing said second amplification duplex with a phage-encoded  
51 RNA polymerase, in the presence of  $Mn^{++}$ , with all four dNTPs, and with those rNTPs  
52 represented in said first spacer sequence to form said sense transcription product.

1 8. The method of claim 7, wherein the method further comprises  
2 repetitively carrying out steps i to vii.

1 9. The method of claim 7, wherein said first or said second spacer  
2 sequence comprises a nucleotide sequence having the formula  $(XY)_n$ ,  
3 wherein n is from 6 to 10,  
4 wherein X and Y are independently selected from the group consisting of an  
5 adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a  
6 thymidine nucleotide,  
7 wherein X and Y are not the same.

1 10. The method of claim 9, wherein X is an adenine nucleotide and Y is a  
2 guanine nucleotide.

1 11. The method of claim 10, wherein n is 9.

1 12. The method of claim 10, wherein the rNTPs are rATP and rGTP.

1 13. The method of claim 7, wherein said first or said second spacer  
2 sequence comprises a nucleotide sequence having the formula  $(X)_n$ ,  
3 wherein n is from 12 to 20,

4 wherein X is selected from the group consisting of an adenine nucleotide, a  
5 guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide.

1 14. The method of claim 13, wherein n is 18.

1 15. The method of claim 7, wherein said sense and antisense transcription  
2 products comprise a nucleic acid strand comprising both ribonucleotides and  
3 deoxyribonucleotides.

1 16. The method of claim 7, wherein said first and said second  
2 amplification duplexes consist of deoxyribonucleotides and ribonucleotides.

1 17. The method of claim 7, wherein said method is carried out at a single  
2 temperature.

1 18. The method of claim 7, wherein said method is carried out at a single  
2 temperature of between 25 °C and 55 °C.

1 19. The method of claim 1, wherein the method is carried out at a single  
2 temperature of greater than 50 °C.

1 20. The method of claim 7, wherein said intermediate duplex comprises a  
2 double-stranded DNA comprising one complete primer sequence followed by the entire  
3 sequence that is to amplified.

1 21. The method of claim 7, wherein said intermediate duplex is formed  
2 from double-stranded DNA, single-stranded DNA, or RNA.

1 22. The method of claim 7, wherein said intermediate duplex is formed by  
2 the process comprising the following steps of:

3 denaturing a double-stranded DNA target to form an upper strand and a lower  
4 strand;

5 hybridizing said first primer to said lower strand to form a first primer-lower  
6 strand complex;

7 extending said first primer-lower strand complex with a Reverse Transcriptase  
8 that lacks RNaseH activity or with a DNA Polymerase to form a first long sense strand  
9 product-lower strand complex;

10 denaturing said first long sense strand product-lower strand complex into a  
11 first long sense strand product and said lower strand;  
12 hybridizing said second primer to said first long sense strand product to form a  
13 second primer-first long sense strand product; and  
14 extending said first primer-first long antisense strand product with a Reverse  
15 Transcriptase that lacks RNaseH activity or with a DNA Polymerase to yield said  
16 intermediate duplex.

1 23. The method of claim 7, wherein said intermediate duplex is formed by  
2 the process comprising the following steps of:  
3 denaturing a double-stranded DNA target to form an upper strand and a lower  
4 strand;  
5 hybridizing said first primer to said lower strand to form a first primer-lower  
6 strand complex;  
7 extending said first primer-lower strand complex with a Reverse Transcriptase  
8 that lacks RNaseH activity or with a DNA Polymerase to form a first long sense strand  
9 product-lower strand complex, wherein said first long sense strand product has a 5' and a 3'  
10 end;  
11 displacing said first sense strand product from said lower strand by:  
12 hybridizing a bumper oligonucleotide to a subsequence on said lower  
13 strand adjacent to said 5' end of said first sense strand product  
14 on the first sense strand product-lower strand complex;  
15 extending said bumper oligonucleotide with a Reverse Transcriptase  
16 that lacks RNaseH activity or with a DNA Polymerase,  
17 thereby displacing said first sense strand product;  
18 hybridizing said second primer to said first long sense strand product to form a  
19 second primer-first long sense strand product; and  
20 extending said first primer-first long antisense strand product with a Reverse  
21 Transcriptase that lacks RNaseH activity or with a DNA Polymerase to yield said  
22 intermediate duplex.

1 24. The method of claim 7, wherein said intermediate duplex is formed by  
2 the process comprising the following steps of:



3                    hybridizing said second primer to a target RNA molecule to form a second  
4 primer-RNA template complex;  
5                    extending said second primer-target RNA molecule complex with a Reverse  
6 Transcriptase that lacks RNaseH activity or a DNA Polymerase to form a first long antisense  
7 strand product-template complex, wherein said first long antisense strand product has a 5'  
8 and a 3' end;  
9                    displacing said first long antisense strand product from said target RNA  
10 molecule by:  
11                    hybridizing a bumper oligonucleotide to a subsequence on said target  
12                    RNA molecule adjacent to said 5' end of said first sense strand  
13                    product on the first sense strand product-lower strand complex;  
14                    extending said bumper oligonucleotide with a Reverse Transcriptase  
15                    that lacks RNaseH activity or with a DNA Polymerase,  
16                    thereby displacing said first long antisense strand product;  
17                    hybridizing said first primer to said first long antisense strand product to form  
18 a first primer-first long antisense strand product complex; and  
19                    extending said first primer-first long antisense strand product with a Reverse  
20 Transcriptase that lacks RNaseH activity or with a DNA Polymerase to yield said  
21 intermediate duplex.

1                    25.    The method of claim 7, wherein said intermediate duplex is formed by  
2 the process comprising the following steps of:  
3                    hybridizing said second primer to a single-stranded target RNA molecule to  
4 form a second primer-RNA template complex;  
5                    extending said second primer-RNA template complex with a Reverse  
6 Transcriptase that lacks RNaseH activity or a DNA Polymerase to form a first long antisense  
7 strand product-template complex;  
8                    denaturing said first long antisense strand product-RNA template complex into  
9 a first long antisense strand product and said single-stranded RNA molecule;  
10                    hybridizing said first primer to said first long antisense strand product to form  
11 a first primer-first long antisense strand product complex; and  
12                    extending said first primer-first long antisense strand product with a Reverse  
13 Transcriptase that lacks RNaseH activity or with a DNA Polymerase to yield said  
14 intermediate duplex.

1                   26.     The method of claim 7, wherein said phage-encoded RNA  
2 polymerase is polymerase selected from the group consisting of : a T7 RNA polymerase,  
3 a T4 RNA polymerase, a T3 RNA polymerase, a SP6 RNA polymerase and a K11 RNA  
4 polymerase.

1                   27.     The method of claim 26, wherein said phage-encoded RNA  
2 polymerase is a mutant phage-encoded RNA polymerase that is competent to incorporate  
3 dNTPs into a template nucleic acid.

1                   28.     The method of claim 27, wherein said phage-encoded RNA  
2 polymerase is a T7 RNA polymerase.

1                   29.     The method of claim 28, wherein said T7 RNA polymerase  
2 contains a Y639F mutation.

1                   30.     The method of claim 28, wherein said T7 RNA polymerase  
2 contains a S641A mutation.

1                   31.     The method of claim 28, wherein said T7 RNA polymerase  
2 contains at least two mutations.

1                   32.     The method of claim 7, wherein said  $Mn^{++}$  is present in a  
2 concentration of between 10  $\mu M$  to 20 mM.

1                   33.     The method of claim 32, wherein said concentration is 10 mM.

1                   34.     The method of claim 7, wherein said target nucleic acid is single-  
2 stranded DNA.

1                   35.     The method of claim 7, wherein the target nucleic acid is  
2 comprised of RNA.

1                   36.     The method of claim 7, further detecting said sense transcription  
2 product, said antisense transcription product, said first amplification duplex, or said  
3 second amplification duplex,  
4                   wherein said detecting comprises hybridizing a detection oligonucleotide  
5 comprising a detectable moiety, wherein said detection oligonucleotide is complementary

6 to a subsequence of said sense transcription product, said antisense transcription product,  
7 said first amplification duplex, or said second amplification duplex.

1 37. A kit for copying a target nucleic acid comprising:  
2 a container containing:  
3 a first nucleotide primer comprising in the following order from 5' to 3':  
4 a phage-encoded RNA polymerase recognition sequence,  
5 a first spacer sequence comprising a sequence of from 12 to 20  
6 nucleotides that consists of one nucleotide type or two  
7 different nucleotide types,  
8 a first target complementary sequence which can bind to a  
9 segment of said target nucleic acid; and  
10 a second primer comprising in the following order from 5' to 3':  
11 a phage-encoded RNA polymerase recognition sequence,  
12 a second spacer sequence comprising a sequence of from 12  
13 to 20 nucleotides that consists of one nucleotide  
14 type or two different nucleotide types, and  
15 a second target complementary sequence which can bind to  
16 a segment of said target nucleic acid.

1 38. The kit of claim 37, wherein said phage-encoded RNA polymerase  
2 is polymerase selected from the group consisting of : a T7 RNA polymerase, a T4 RNA  
3 polymerase, a T3 RNA polymerase, a SP6 RNA polymerase and a K11 RNA polymerase.

1 39. The kit of claim 38, wherein said phage-encoded RNA polymerase  
2 is a mutant phage-encoded RNA polymerase that is competent to incorporate dNTPs into  
3 a template nucleic acid.

1 40. The kit of claim 38, wherein said phage-encoded RNA polymerase  
2 is a T7 RNA polymerase.

1 41. The kit of claim 40, wherein said T7 RNA polymerase contains a  
2 Y639F mutation.

1 42. The kit of claim 40, wherein said T7 RNA polymerase contains a  
2 S641A mutation.



1                   43.     The kit of claim 40, wherein said T7 RNA polymerase contains at least  
2 two mutations.

1                   44.     The kit of claim 37, further comprising a member selected from the  
2 group consisting of:

3                   a DNA polymerase;

4                   a Reverse Transcriptase that lacks RNaseH activity;

5                   a phage-encoded RNA polymerase;

6                   all four dNTPs;

7                   those rNTPs represented in said first and second spacer sequences;

8                   reaction buffer containing manganese in a concentration from 10  $\mu$ M to 20

9                   mM;

10                  a positive control target nucleic acid; and

11                  instructions for carrying out a method of copying a nucleic acid using said first  
12 primer and said second primer.